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THE PATHOGENESIS OF RADIATION INJURIES AND REPAIR PROCESSES IN RAT EMBRYOS AFTER THE IRRADIATION OF THE FEMALES ON THE 10TH DAY OF PREGNANCY

by Ye.A. Val'shtrem

[Following is the translation of a paper by Ye.A. Val'shtrem entitled "Patogenez luchevykh povrezhdeniy i reparativnye protsessy v zarodyshakh krys posle obluchcniya samok rentgenovymi luchami na 10-y den Beremennosti" (English version above) in Arkhiv Anatomii, Gistologii i Embriologii (Archives of Anatomy, Histology and Embryology), Vol. 38, No. 3,1960, Leningrad, pages 72-79]

A number of papers on the action of ionizing radiations on embryonic development of mammals (L. Russell 1950, L. Russell and W. Russell 1952 and 1954, E. G. Lomovskaya and Ye.I. Vorob'eva 1957, Yu.M. Olenov and

A.D. Pushnitsyna 1952, P.G. Svetlov and G.F. Korsakova 1954, and others). It has been established that radiosensitivity of embryos at the end of embryogenesis decreases, the character of anomalies caused by irradiation changes also, but in embryogenesis there are particular critical developmental periods, in which under the influence of radiation anomalies of separation conceptions appear and others. In studying the effect of different dosages of X-rays on mammalian embryogenesis, the radiation is usually made in different pregnancy periods, and the results are considered at the end of pregnancy. But another experimental set-up could facilitate the understanding of the injury mechanism appearing with irradiation, and namely: irradiation in any one definite developmental stage with the consideration of results in different periods after the influence. Thus, it would be possible to trace the formation and development of the anomalies which occur. This kind of study was made in this paper.

Study method.

According to the unpublished data in the Embryology

Laboratory of P.G. Svetlov and G.F. Korsakova, on the tenth day of pregnancy the establishment of embryonic rudiments occurs in rats and in this critical period in the pregnant rats the irradiation with a dosage of 100 to 250 r invariably causes damage of local character, in the form of anomalous development of different organs, mainly different areas of the head (encephalia, microcephalia, anophthalmia, microphthalmia, cleft palate and others), and also of general character (adema, hematimus, small body sizes and so on). Therefore in our experiments we applied a single total-body irradiation of the female rats precisely on the tenth day of pregnancy. The 10th day of rat embryogenesis is also the beginning of the critical period for placental development (P.G. Svetlov and G.F. Korsakova 1954).

In this developmental period the placental rudiments are especially easily damaged, for example by overheating and introduction of narcotics, and in addition to this, the formation of anomalies of fetuses development is the consequence of pathological changes in the developing placenta (E.G. Svetlov and G.F.Korsakova

1954). Therefore our attention was also turned to the placental development.

Two series of experiments were made with irradiation of animals at dosages of 130 and 240 r. These dosages were selected as average in intensity, and as the ones best studied with respect to the final result of the ionizing radiation effect. Irradiation was made with the equipment RUM-3 with foosl distance 40 cm, voltage 180 kv, current 15 ma; filters - 0.5 mm, copper and 1.0 mm Al; rate 30r/minute. Registration of damage was made daily with the beginning of 11th up to 17th developmental day inclusive. The rats were cut open under ether narcosis, and studies of the embryos and placentas were made during life and later they were fixed by Bovin's and Carnoy's fluids, were embedded in parafin and histologic studies of embryos were made on the stained sections. We studied 1,143 embryos, 607 of them were experimental and 536 control.

As is known, in the rat embryo on 10th day of development the anterior end is bulged, the rudiment of the nervous system has the form of wide medullary plate with a groove in the middle; the body is not yet segmented,

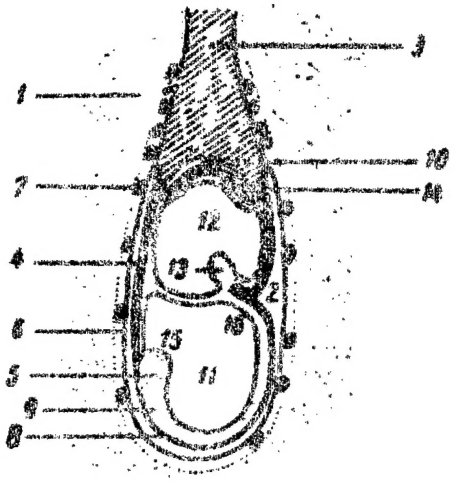


Fig. 1. Longitudinal section through normal rat embryo on 10th day of development after fertilization (according to P.G. Svetlov and G.F. Korsakova 1954).

1 - Lycunas in the decidual tissue; 2 - Vitelline sac; 3 - Ectoplacentar cone; 4 - Vitelline endoderm; 5 - Ectoderm; 6 - Reichert's membranes; 7 - Gigantic trophoblast cells; 8 - Intestinal endoderm; 9 - Mesoderm ; 10 - Ectoplacentar cone cavity; 11 - Ammionic cavity; 12 - Exocoelom; 13 -Allantois; 14 - Chorial plate; 15 - Head end of the embryo; 16 - Tail end of the embryo.

The future placenta is represented by two rudiments: the chorion plate and allantois. The chorion plate is presented by the cavity wall of active placenta cone, at this stage of development having a form of a slot, turned to exocoelom. It consists of cells which lie close to each other and are distributed in many layers, and which in the future give rise to the beginning of placenta labyrinth trophoblast. Allantois in this stage of development represents a protruding mesenchyme covered by peritoneal epethelium which grows in the direction of chorial plate, but at the 10th day of embryic development is still not touching it (Fig. 1).

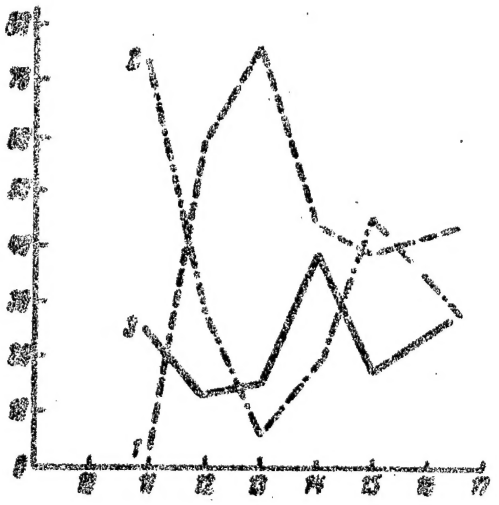


Fig. 2. Distribution curve of normal, anomalous and dead

embryos according to the days of development after X-irradiation according to the days of development after X-irradiation with 130 r.

Horizontal - dates of embryonic development; vertical - number of embryos in %. 1 - Anomalous, 2 - normal, 3 - dead embryos.

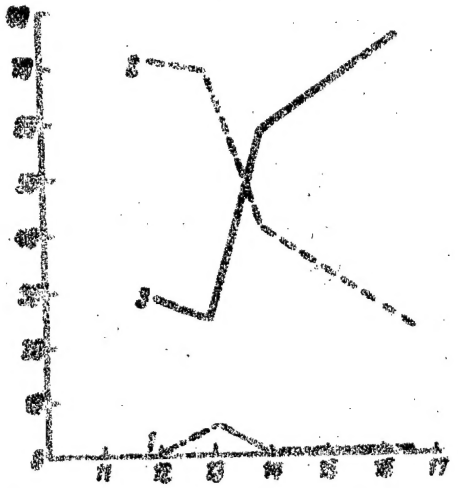


Fig. 3. Distribution curve of normal, anomalous and dead embryos according to the days of development after X-irradiation with 240 r.

Horizontal - days of embryonic development; vertical - number of embryos in %. 1 - Normal, 2 - anomalous, 3 - dead embryos.

Our own observations. The results of irradiation effect are given in Table 1.

As it is seen from Table 1, irradiation with dosage 130 r led to the destruction of 23.5% of the total number of embryos at the end of pregnancy, while in the control the death rate of embryos was 8 to 12%. The mentioned dosage causes a large difference of embryo development anomalies, the total number of which reaches up to 45.45%. In the controls these anomalies were rare - 0.18%, i.e., one case per 536 embryos.

The distribution of normal, dead and anomalous embryos by days of development in the given series of experiments are given in Table 2 and on Fig. 2.

After one day off irradiation the number of anomalou embryos is small - 2.77%, but in the subbequent days it increases, reaching on the 13th day the largest number 75.5%. Further the number of anomalous embryos decreases and is less than half of the total number of embryos, 38.9 to 43.3%. The curve of embryo destruction passes much lowe than the anomaly curve (see Fig. 2). On the following day after irradiation the embryo death reaches 25%. Then in

Type of experiment	Total no. embryos	Normal embryos in (in %)	Live anomalous embryos (in %)	Dead embryos (in %)
Control.....	536	87,82-91,82	0.18	8.00-12.00
Irradiation with 130 r.....	308	31.49	45.45	23.06
Irradiation with 240 r.....	299	1.33	42.82	55.85

Table 1. Irradiation results.

Embryos	Days of development after fertilization						Total embryos
	11	12	13	14	15	16-17	
Normal.....	26 (72,3%)	13 (26,9%)	5 (9,4%)	10 (13,2%)	22 (44,1%)	17 (28,3%)	97 (31,49%)
Anomalous....	1 (2,77%)	26 (57,7%)	40 (75,5%)	34 (43,6%)	23 (38,9%)	26 (43,3%)	140 (45,45%)
Dead	9 (25%)	6 (13,3%)	8 (15,1%)	31 (39,2%)	10 (17%)	17 (28,3%)	71 (23,05%)

Table 2. Number of normal anomalous and dead embryos according to days of development after irradiation with 130 r.

the following days the number of dead embryos decreases to 13 to 15%, increasing on the 14th day to 38.2%. The percentage increase of dead embryos on the 14th day probabl occurs to a considerable degree at the expense of the dead anomalous embryos, since the number of the later this day sharply decreases. But the percentage increase of dead embryos in the last observational days also occurs at the expense of embryos, which up to this time had a normal form, since the percentage of anomalous embryos does not decrease in comparison with the previous day, and even increases somewhat. On the next day after irradiation the percent of normal embryos is 72.2% and decreasing in the following days to 9.4% (on the 13th day) again increases later up to 44.1% on the 15th day.

Analysis of the numerical data given in Table 2 and of the curves represented in Fig. 2 permits consideration of the cause of embryo death on the following days after irradiation as not due to their anatomical anomalies. On the 11th day of pregnancy, when death of 25% of embryos occurs, there are almost no embryos with anomalous structures, there are little more than in the control: 2.77% (control-0.18% see Table 1). The under-development

of the placentas cannot be the cause of the death in this case either as in the embryogenesis periods the placenta is not yet functioning. Evidently, embryo deaths in the first days after irradiation is caused by the direct influence of X-rays.

Anomalies in embryos are not detected immediately after irradiation: their greatest number is on the 13th day of development (75.5%), and then their number decreases (43.6% to 38.9%). This fact can be explained either that the embryos, which became anomalous on the 13th day, die in large number, or that the development of anomalies are repaired.

The percentage increase of anomalous embryos can be only at the expense of the display of pathogenetic irradiation effect, made earlier. Its decrease can be either because of the embryo's death, or the cause of their repair (i.e., the conversion of anomalous embryos into the headings "dead" and "normal"). The comparison of curves on Fig. 2 shows that it is impossible to explain the percentage decrease of anomalous embryos on the 14th and subsequent days of development only by the death of these embryos. Although the percentage of dead embryos

increases on the 14th day of development in comparison with 13th day (by 23.1%), but it does not increase sufficiently to explain the percentage decrease of anomalous embryos on this day of development (by 3.19%). The number of normal embryos however considerably increases on the 14th day of development (by 8.8%), and in the future continues to increase, reaching 41.4% on the 15th day of development. Thus, the curves of normal, anomalous and dead embryo numbers undoubtedly show that after irradiation of the rats on the 10th day of pregnancy with a dosage of 130 r, a considerable amount of the obtained damages is repaired. There are indications in the literature on repair in embryos after injuring them with X-rays (A.A. Zanarzii, V.Ya.Aleksandrov, G.S. Strelin and G.V. Yasvoin, 1936; Yu.M. Olenov and A.D. Pushnitsyna 1952 and others). High regenerative ability is a general characteristic for the embryos.

Irradiation of animals on the 10th day of pregnency with a 240 r dose caused a considerably larger percentage of embryo deaths (55.85%, see Table 1). The number of anomalous embryos is also very high (42.82%). From the

299 embryos studied by us only four were normal, which is only 1.33%.

The distribution of normal, anomalous and dead embryos according to days development after fertilization is represented in Fig. 3 and in Table 3.

Embryos	Days of development after fertilization						Total Embryos
	11	12	13	14	15	16-17	
Normal.....	—	0	3 (5,74%)	0	0	1 (0,8%)	4 (1,33%)
Anomalous..	—	30 (71,4%)	31 (69,7%)	22 (11,5%)	17 (34,7%)	25 (23,7%)	125 (42,82%)
Dead.....	—	12 (28,6%)	13 (24,5%)	31 (58,5%)	32 (65,3%)	80 (75,5%)	167 (56,85%)

Table 3. Number of normal, anomalous and dead embryos according to the days of development after X-irradiation with 240 r.

Maximum percentage of anomalous embryos was observed even on the 12th development day - 71.4%. It remains almost the same on the 13th day as well, but beginning with 14th development day it sharply decreases to 23.7% (on 16th to 17th day). The percentage curve of dead embryos, on the contrary, increases: from 28.6 and 24.5% on 12th and 13th day to 75.5% on 14th day of

development. The curve of normal embryos is about zero for all observational periods.

The relation between anomalous and dead embryo numbers at irradiation with 240 r turns out to be very simple: the percentage curves of anomalous and dead embryos are symetrical, and each point of one curve practically supplements the corresponding point in the other curve up to 200%. This shows, that beginning from 14th day of development (4th day after irradiation) percentage increase of dead embryos occurs because of the death of anomalous embryos. The possibility is not excluded that also in this case there are some regulation effects in anomalous embryos, which remain alive to the 17th day of development, but quantitative relations of anomalous and dead fetuses do not give any hints of this. It can be only assumed, that if there are any regulative effects in this case, that they are very insignificant and do not bring anomalous embryos to normal conditions.

The anomaly development characteristic of embryos after the irradiation of females on the 10th day of pregnancy is extremely diversified. In this report data will be given only for an irradiation of 130 r.

Observations were begun with the third day after irradiation (12th day of development). In the norm, the 12th day embryo has a segmented body and a very long tail. The hemispheres of the brain are in the form of two bulbs. They are in the buds of two lymph pairs. The two-chambered heart is pulsating. Vascular arcs are clearly seen; in irradiated animals however general underdevelopment is noticed primarily. The hemispheres of the brain have a changed configuration, and small size. The vascular arcs are not developed. On the 4th day after irradiation (13th day of pregnancy) the embryos usually very much lack development. Also, underdevelopment of brain and the front part of the skull are characteristic for them.

By the 15th day the normal embryos are already formed. The head is well separated, and all parts of the body are developed. The buds of whiskers and bristles are seen. Digits are marked in the limbs. In 15 day experimental embryos (6th day after irradiation), the effects of brain and jaw development anomalies remain. The eyes are not seen from outside. The body sizes are usually smaller than the norms. In 16 day old irradiated embryos,

very deep anomalies of head-brain can be observed, all of the sections of which are mixed together, the maxillary defects and the eye underdevelopment remain, if the embryos do not reach normal sizes (Fig. 4a and b), i.e., by this day the development of anomalies acquire their final character.

The following anomalies were most frequently encountered in the irradiated experimental animals: 1 - the effects of eye development (their underdevelopment and absence) - 120 cases. 2 - development effects of front sections of head-brain (underdevelopment and anomalies) - 78 cases. 3 - underdevelopment of maxillae - 38 cases. Other forms of embryo defects (anomalies of limbs and the back part of the body, general under development, heart ectopy and others) were met in single cases.

Thus, as the above mentioned anomalies show (typical for rat embryos irradiated on the 10th day of development) the diseases appearing are already clearly noted on the 3rd day after irradiation, and in the future they are deepened.

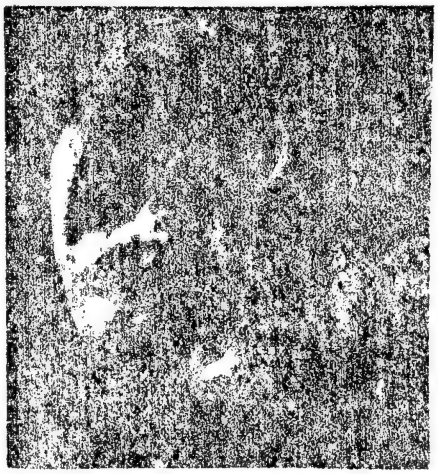
Special interest is presented by the developmental

anomalies of allantois which are detected beginning with the 12th day of development. As it was said above, at the moment of embryo irradiation on 10th day of development, the trophoblastic and allatoidal rudiments of placenta are still not connected with each other, but allatoidal outgrowth already jumps deeply into the exocoelom in the form of a tongue (see Fig. 1). In the many irradiated embryos the allantois does not grow to the chorial plate and there is no connection of these two placenta buds. The allantois bud remains in the form of a free outgrowth on the dorsal side of the body, whereupon its distal end is bowed, being pyriform (see Fig. 5a and b). With 130 r irradiation this anomaly type is encountered in 2.9% of embryos, and with 240 r dosage - in 14.7% of embryos. Thus, the allantois turns out to be a part of embryo which is very sensitive to irradiation. Naturally, if this most important bud of the placenta labryinth stops in the development and does not coalesce with chorial plate, then neither the placenta nor the umbilical cord can form in such embryos. These embryos remain alive for sometime and even develop, but then of course die. After the 14th day these embryos cannot be observed. The

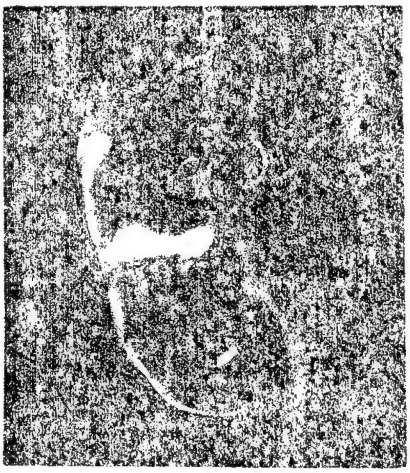
mesenchyme of allantois which on the 10th day stopped in its development, on the 14th day of embryonic development has a typical structure; its peripheral part is covered by mesothelium. The mesenchyme is well vascularized; there is a large number of erythroblast in the vessels. However cellular necrosis and products of decay are observed in the mesenchyme.

Even if by the 14th day of development after fertilization there is no connection of the allantois with chorial trophoblasts, then the trophoblast cells in the region, where the labyrinth should have been formed, become friable. The viability of prophoblasts in this case is considerably greater than in the norm, but the growth of the labyrinth itself in such placenta delayed and by its dimensions it corresponds to placenta labyrinths of earlier development periods. The myterses in labyrinth prophoblasts are encountered much more rarely, than in normal embryos. Anatomically however the placenta is normally formed, like a genetic depot is well developed.

The marked disruptions of allantoic development make probable the assumption, that in the cases, when

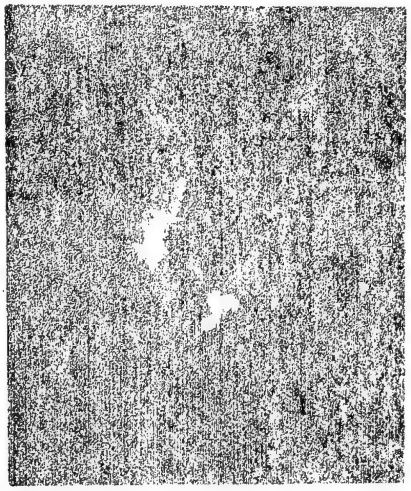


a.



b.

Fig. 4. Normal embryo on the 16th day of development after fertilization (a), and an embryo of the same developmental period after irradiation with 130 r on the 10th day of embryogenesis (b).



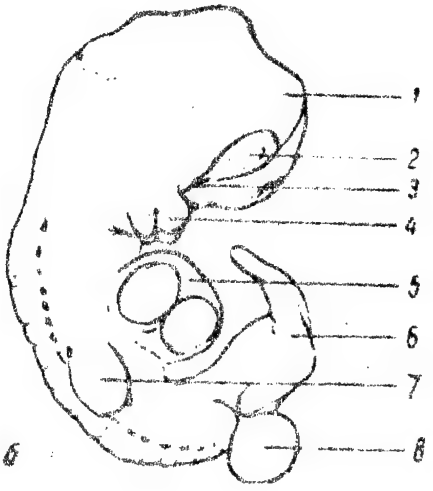


Fig. 5. Irradiated embryo on the 14th day of development. The pyriform allatois are on the dorsal side of the body.

a - photograph, b - drawing. 1 - large hemispheres of the brain; 2 - olifactory pit; 3 - upper maxilla; 4 - lower maxilla; 5 - heart; 6 - hind limbs; 7 - fore limbs; 8 - allantois.

the connection of allantois with the chorial plate is accomplished, the further development of placenta labyrinth still turns out to be lower because of the damage of allatois mesenchyme as one of the most sensitive rudiments of embryo in this development stage.

The study of placentas in irradiated pregnant females present an exceptional interest, as this can explain many things in the problem of irradiation damages. This study is being made by us at present and will make the contents of next report.

Conclusions

1. After single total-body irradiation of female rats on the 10th day of pregnancy by X-rays with a 130 r dose, anatomical anomalies (easily discovered even with external examination of embryo - underdevelopment of brain, eyes, vascular arcs), can be repaired.

2. The stated developmental anomalies are discovered on the 2nd and 3rd day after irradiation and in the future (if they are not repaired deviations from the norm become more pronounced.

3. After the single total-body irradiation of female rats on the 10th day of pregnancy by X-rays with a

240 r dose repair effects are not observed through external examination of embryos.

4. The allantoic placenta rudiment on the 10th day of pregnancy possesses a very high radiosensitivity. Owing to this in a number of cases in the fetuses of the experimental animals, the allantoic placenta rudiment does not coalesce with the prophoblast rudiment, because of which neither the placenta labyrinth nor the umbilical cord can be formed, which is observed by applying dosages of 240 r and also 130 r.

5. The high radiosensitivity of allantoic placenta rudiment in the critical period of its development makes possible the assumption, that in the pathogenesis of irradiation damages of embryos, the anomalies of placenta development have a great importance.

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PATHOGENESIS OF IRRADIATION HAZARDS AND REPARATIVE PROCESSES IN RAT EMBRYOS AFTER *X*-RAYING OF RAT FEMALES ON THE 10th DAY OF PREGNANCY

E. A. Walshtrem

The effect produced upon the development of rat embryos by total irradiation of rat females on the 10th day of pregnancy was studied. The application of the irradiation dose equal to 130 *r* caused anatomical anomalies well observed even during external examination of embryos on the 12th—13th days of the embryogenesis (under development of brain, eye, visceral arcs). The anomalies may partially reparate by the 17th day of development. The application of x-rays in dose equal to 250 *r* intensified deviations from the norm; the external examination did not reveal any reparative phenomena.

Allantoid anlage of placenta on the 10th day of pregnancy is exceedingly sensitive to radioactivity. After the application of the foregoing irradiation doses to the animals under study it was observed in a number of cases that the allantoid anlage of placenta did not coalesce with the trephoblast one. Due to that neither the labyrinth of placenta nor umbilical cord could be formed, that made any further development of the embryo impossible.

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